<u>MHC class II haplotype specific immunodominancy of peptides derived from RSV</u>
<u>Fusion (F) and Attachment (G) proteins</u>

5   <u>Field of the invention</u>

The present invention relates immunodominant peptides derived from human respiratory syncytial virus (H-RSV) that may be used in *ex vivo* diagnosis of immune responses to H-RSV, preferably in a MHC class II haplotype specific manner. The immunodominant peptides are preferably derived from the H-RSV

10   Fusion (F) and Attachment (G) proteins. The invention further relates to the use of these immunodominant H-RSV-derived peptides for vaccination against H-RSV, preferably in a MHC class II haplotype specific manner.

<u>Background of the invention</u>

15   Human respiratory syncytial virus (H-RSV), classified in the genus Pneumoviruses of the family of Paramyxoviridae, is a major cause of lower respiratory disease in young infants, immunocompromised individuals and the elderly. No safe and effective licensed H-RSV vaccine is currently available. In an early vaccine trial with a formalin inactivated alum-precipitated H-RSV vaccine (FI-

20   RSV) given intramuscularly, an enhancement of disease occurred in vaccinees upon subsequent exposure to the natural virus. This enhanced illness was characterised by bronchiolitis, hypoxemia and pneumonia and infiltration into the lungs of lymphocytes, neutrophils and some eosinophils. This cellular infiltration suggested that immune-mediated injury can contribute to the pathogenicity of RSV disease.

25   Primary infection with RSV can cause lower respiratory tract disease in young infants manifesting as a pneumonia or bronchiolitis. The disease is associated with the inflammatory response to infection likely involving the production of cytokines and chemokines by lung epithelial cells and immune cells that are recruited to the lungs. The T cell response is an essential component of the immune response needed

30   for viral clearance from the lungs (Alwan et al., 1992, Clin. Exp. Immunol. 8: 527-536; Graham et al., 1991, J.Clin. Invest. 88:1026-1033; Fishaut M. et al., 1980, J. Pediatr. 96:179-186). Antibodies against F and G proteins are generated during RSV

infection but even in the presence of high levels of virus neutralising antibodies reinfections occur (Hall et al., 1991, J. Inf. Dis. 163: 693-698) and antibodies are not needed for viral clearance (Graham et al., 1991, J. Virol. 65: 4936-4942). Also, vaccination with the FI-RSV vaccine induced high titres of RSV specific antibodies

5   in vaccinees, yet caused more severe clinical disease upon natural infection. While in RSV infections T cell responses are required for clearance of RSV from the lung it has been shown in murine studies that CD4 as well as CD8 T cells can be responsible for enhanced lung pathology (Cannon et al., 1988, J.Exp. Med. 168:1163-1168; Alwan et al., 1994, J. Exp. Med. 179: 81-89). Severe pneumonia

10   with extensive influx of eosinophils into the lungs can be elicited in mice by Th2 cells specific for RSV-G, that are primed during vaccination with FI-RSV before intra-nasal challenge with life RS-virus (Waris et al., 1996, J. Virol. 70: 2852-2860; Openshaw et al., 1992, Int. Immunol. 4: 493-500). This strong eosinophilic inflammation is also observed after RSV challenge of mice vaccinated with a

15   vaccinia recombinant expressing solely the G protein of RSV. One peptide corresponding to residues 183-195 in the G protein of RSV appears to be recognised by Balb/c specific Th2 cells (Varga et al., 2000, J. Immunol. 165: 6487-6495). CD8 T cell responses against epitopes derived from G protein have not been observed in BALB/c mice nor in man. CD4 T cells in mice vaccinated with a vaccinia

20   recombinant expressing the F protein of RSV that are subsequently challenged with life virus are Th1 (Alwan et al., 1993, J. Immunol. 150: 5211-5218). CD8 T cell responses against F are common in mice of different MHC types (Chang et al., 2001, J. Immunol. 167:4254-4260). In the Balb/c model a CD8 T cell response has been shown to regulate the outcome of CD4 T cell responses preventing enhanced disease

25   (Srikiatkhachorn and Braciale, 1997, J. Exp. Med. 186: 421-432). Whether the insights obtained from the murine model can be translated to the human situation has to be evaluated.

        In PBMC's from healthy adults as well as from diseased infants, CD8 T cell responses and CD4 T cell responses can be visualised. Some information on viral

30   protein/peptide recognition by human CD4 T cells and CD8 T cells has been uncovered in the art. E.g. in Levely et al. (1991, J. Virol. 65: 3789-3796) CD4 T cell responses against some subdomains of the F protein have been described using T

cell proliferation assays. A dominant antigenic peptide was characterised encompassing amino acid residues 338-355 of the F protein. The response against this peptide was HLA-DR restricted, but the presenting MHC molecule was not further identified. Moreover, the peptide was recognised by different donors that did not share one single MHC class II molecule. Goulder et al. (2000, J. Virol. 74: 7694-7697) describe an H-RSV specific cytotoxic T cell epitope that is HLA class I (HLA B7) restricted and that is derived from the H-RSV nucleoprotein. Brandenburg et al. (2000, J. Virol 74: 10240-10244) describe HLA class I restricted cytotoxic T cell epitopes derived from the H-RSV F protein. A peptide spanning amino acids 118 - 126 of H-RSV F protein proved to be HLA B57 restricted in one patient and a peptide spanning amino acids 551 - 559 of H-RSV F protein proved to be HLA C12 restricted in another patient.

There is, however, still a need for further characterisation of the MHC class II haplotype specific immunodominancy of peptides derived from the F and G proteins of RSV. Such detailed knowledge about antigenic peptides may be used for the development of subunit vaccines and it may further enable the use of tools, like MHC class II tetramers, to monitor *ex vivo* virus specific immune responses in infected patients or evaluate correlates of protection in vaccinated individuals.

                              Description of the invention
The present invention is based on the discovery and characterisation of immunodominant peptides derived from the Fusion (F) and Attachment (G) proteins of H-RSV that are recognised by autologous CD4 T cells in the context of a panel of MHC class II molecules that are expressed most frequently within the Caucasian population. Antigenic peptides are found in both G and F protein. In most donors, CD4 cells recognise more than one peptide within the F protein. Several peptide antigens are productively presented in the context of more than one MHC class II molecule, while at the level of detection of the direct elispot assays performed, there are also peptides that are MHC class II haplotype specific. Most peptides are presented by HLA DR molecules. In general donors that share MHC class II molecules recognise the same pattern of peptides. The present invention thus relates

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to diagnostic, prophylactic and therapeutic methods that are based on the MHC class II haplotype specific antigenicity of H-RSV F or G protein-derived peptides.

In a first aspect the invention relates to method for *ex vivo* diagnosis of MHC class II haplotype specific immune responses to H-RSV antigens in a subject. The method comprises the steps of: (a) determining the MHC class II haplotype of the subject; (b) providing a composition comprising peripheral blood mononuclear cells (PBMC's) from the subject; (c) mixing the composition comprising PBMC's with a peptide comprising at least 9 contiguous amino acids from an amino acid sequence selected from Table 1 that matches the MHC class II haplotype of the subject in accordance with Table 1; and, (d) determining the response of the PBMC's to the peptide.

In the method, the MHC class II haplotype of the subject is determined using any one of a number of methods well known in the art, (see e.g. Coligan et al., 1994, In: Coico R, ed. Current protocols in immunology. Vol. 2: John Wiley & Sons, Inc., Chapter 7: Immunologic studies in humans). Similarly, the composition comprising PBMC's is obtained from the (human) subject using a variety of methods well known in the art (see e.g. Coligan et al., 1994, In: Coico R, ed. Current protocols in immunology. Vol. 2: John Wiley & Sons, Inc.,: 711-712). Blood samples are usually processed to remove erythrocytes and platelets (e.g., by aphaeresis, Ficoll density gradient centrifugation and/or red blood cell lysis or other such methods known to one of skill in the art) and the remaining PBMC sample, which includes the T-cells of interest, as well as B-cells, macrophages and dendritic cells, may be used directly in the assay. The composition comprising PBMC's may e.g. consist of the PBMC bulk that is obtainable by from blood obtained from the subject. For higher yields of PBMCs, the subject from whom the PBMCs are obtained may be given a pre-treatment with GM-CSF for mobilising mononuclear cell subpopulations. The PBMC composition may further be enriched for specific subsets of mononuclear cells, preferably T cells, more preferably CD4$^+$ T cells. The PBMC composition may be enriched for T cells, or in particular for CD4$^+$ T cells, by methods known in the art, such e.g. by expansion of T cells or CD4$^+$ T cells as described e.g. in Coligan et al. (1994, In: Coico R, ed. Current protocols in immunology. Vol. 2: John Wiley & Sons, Inc., 1994: 711-94).

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In step (c) of the diagnostic methods of the invention a selected peptide derived from the H-RSV F and/or G proteins is mixed with the composition comprising the PBMC's. The H-RSV F or G protein-derived peptides of the invention may be obtained as described below. The peptide to be mixed with the

5   composition comprising the PBMC's of a given subject are selected to match the MHC class II haplotype of the subject. Table 1 summarises for each of the major MHC class II haplotypes that are prevalent in the caucasian population, the H-RSV F and G protein derived peptides that are recognised by the particular MHC class II haplotype, as measured by IFN-γ production in a direct elispot assay. Thus, in the

10   method of the invention, subsequent to having established the MHC class II haplotype of a subject, the composition comprising the PBMC's form the a subject is brought into contact with one or more (poly)peptides that comprise an amino acid sequence of at least 9, 10, 11 or 12 contiguous amino acids from an amino acid sequence listed in Table 1 as being recognised by the MHC class II haplotype of the

15   subject. Peptides and polypeptides of the invention are further described herein below. Preferably the peptides are brought into contact with the composition comprising the PBMC's at a concentration that ranges from 1 nM to 100 μM, more preferably 10 nM to 50 μM, most preferably 100 nM to 10 μM. The amount of PBMC's to be brought into contact with the peptide depends on the assay format

20   used for detecting the PBMC response. Various such assays are described below and the amount of PBMC's to be used in these assays are known to the skilled person.

Finally, in step (d) of the method, the response of the PBMC's to the H-RSV antigenic peptide(s) is determined. The antigen specific response of the PBMC's may be determined using a number of methods available in the art (see e.g. Coligan

25   et al., 1994, In: Coico R, ed. Current protocols in immunology. Vol. 2: John Wiley & Sons, Inc., Chapter 7: Immunologic studies in humans). Preferably, the antigen specific response of the PBMC's is determined by determining the proliferation of the PBMC's in response to the presence of the H-RSV antigenic peptides. More preferably, the proliferation of T cells in response to the presence of the H-RSV

30   peptide(s) is determined. The proliferation of T cells in response to the presence of the H-RSV peptide(s) is preferably determined directly in the composition comprising the PBMC's, i.e. without pre-expansion of the T cells. Alternatively, the

T cells or more specifically, the CD4$^+$ T cells may be enriched or pre-expanded to increase the sensitivity of the diagnostic method of the invention. In a further preferred method, the proliferation of CD4$^+$ T cells (in the composition comprising PBMC's) in response to the presence of the H-RSV peptide(s) is determined. The

5   proliferation or response of the CD4$^+$ T cells may be determined using a variety of methods available in the art , (see e.g. Coligan et al., 1994, In: Coico R, ed. Current protocols in immunology. Vol. 2: John Wiley & Sons, Inc., Chapter 7: Immunologic studies in humans). In a preferred method the proliferation or response of the PBMC's, in particular the CD4$^+$ T cells is determined by measuring the

10   concentration of a "soluble protein factor" that is secreted by a PBMC or T-cell in response to antigenic stimulation. A variety of secreted soluble protein factors can be detected by the assays disclosed herein. The soluble factors may be cytokines, lymphokines or chemokines. Typically this secreted factor is a lymphokine, such as enumerated below. As a result of the increased sensitivity of the assay, factors

15   secreted by rare T-cells which occur in low frequency can be detected. Factors which are detected by this method include, but are not limited to lymphokines, cytokines and chemokines such as for example, IFN-γ, TNF-α, IL-2, IL-3, IL-4, IL-5, IL-10, IL-13, TGF-β, RANTES, and GM-CSF.

       A variety of assay formats can be used to detect the increased levels of

20   secreted factors produced by the assay described herein. Suitable assays include both solid phase (heterogeneous) and non-solid phase (homogeneous) protocols. The assays can be run using competitive or non-competitive formats, and using a wide variety of labels, such as radioisotopes, enzymes, fluorescers, chemiluminescers, spin labels, and the like. Such methods include, but are not limited to enzyme-linked

25   immunosorbent assays (ELISA), both direct and reverse formats, and other solid phase assays. It will be recognised that negative controls, i.e., samples run without added antigen, and positive controls, i.e., samples run with antigens, such as tetanus toxoid, known to elicit lymphokine secretion from T-cells will be run as necessary under otherwise duplicative conditions to validate the assay results.

30   Some assays rely on heterogeneous protocols where a ligand complementary to the secreted factor (such as antibody against the secreted factor) is bound to a solid phase which is used to capture the secreted factor. The ligand may be

conveniently immobilised on a variety of solid phases, such as dipsticks, particulates, microspheres, magnetic particles, test tubes, microtiter wells, and plastics, nitrocellulose or nylon membranes and the like, including polyvinyl difluoride (PVDF) (e.g., 96 well plate with a PVDF membrane base (Millipore

5   MAIPS45-10))and ELISA grade plastic. The captured factor can then be detected using the non-competitive "sandwich" technique where a directly or indirectly labelled second ligand for the factor is exposed to the washed solid phase. Such assay techniques are well known and well described in both the patent and scientific literature. See, e.g., U.S. Pat. Nos. 3,791,932; 3,817,837; 3,839,153; 3,850,752;

10   3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; and 4,098,876. Enzyme-linked immunosorbent assay (ELISA) methods are described in detail in U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,879,262; and 4,034,074. ELISA assays detect very low titres of secreted factors. Also see, "Enzyme immunohistochemistry" in Practice and Theory

15   of Enzyme Immunoassays, P. Tijssen (Elsevier 1985).

A commonly used assay format is the antibody capture assay. The general protocol is simple: a ligand, e.g., an unlabeled antibody for the secreted factor, is immobilised on a solid phase, and the secreted factor is allowed to bind to the immobilised antibody. The bound secreted factor is then detected by using a labelled

20   secondary reagent that will specifically bind to the captured factor ("direct sandwich assay"). Alternatively, the secondary reagent will not be labelled, but will be detected by subsequent binding to labelled tertiary binding reagent complementary to the second binding reagent ("indirect sandwich assay"). The strength of signal from the bound label allows the determination of the amount of secreted factor

25   present in the sample and this in turn allows the quantification of the number of activated T-cells present in the sample. As will be recognised by the skilled person, the sandwich assay can be used to detect any secreted factor which has two epitopes, each of which can be recognised by the specific binding pair members. Choosing appropriate capture and detection antibody pairs permits the application of this assay

30   to the detection of T cells secreting a variety of soluble factors. A list of antibody pairs which can be used in this assay is inter alia presented in U.S. Pat. No. 6,218,132.

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Alternatively, the proliferation or response of the T cells may be determined by intracellular cytokine staining as e.g. described by Murali-Krishna et al. (1998, Immunity $\underline{8}$: 177-187).

A preferred method for determining the proliferation or response of the $CD4^+$

5   T cells comprises measuring the IFN-γ production by the $CD4^+$ T cells in response to the presence of the H-RSV peptide(s). Again, methods for measuring antigen induced IFN-γ production by the $CD4^+$ T cells are well known in the art (see e.g. Coligan et al., 1994, In: Coico R, ed. Current protocols in immunology. Vol. 2: John Wiley & Sons, Inc., Chapter 6: Cytokines)., and include e.g. a method wherein IFN-

10   γ production is measured in a elispot assay, as described in the Examples herein. Preferably, the IFN-γ production is measured in a direct elispot assay, whereby "direct" is understood to mean directly on the composition of PBMC's obtained from the subject, without pre-expansion of the T cells or $CD4^+$ T cells. Likewise, the other above-mentioned lymphokines and cytokines may be measured in a direct elispot

15   assay.

The diagnostic methods of the invention may be used for a variety of reasons on different subjects. The methods may e.g. be used on a subject undergoing a lower respiratory tract (LRT) disease as manifested by pneumonia and/or bronchitis. In such subjects, the method may be used to determine the involvement of H-RSV in

20   the LRT disease, e.g. to distinguish between infections with H-RSV, influenza virus and/or human pneumovirus. Having established the diagnosis of an H-RSV infection in a given subject, the diagnostic methods of the invention may further be used to monitor the immune response to H-RSV antigens in the subject undergoing an H-RSV infection. The methods may further be used to determine the status of a

25   subject's immune response to H-RSV in a subject having undergone an infection with H-RSV, i.e. in a subject that no longer shows any clinical symptoms of H-RSV infection. Finally, the methods of the invention may be used to determine the status of a subject's immune response to H-RSV in a subject having been vaccinated against H-RSV. In particular, the methods may be used to evaluate correlates of

30   protection in individuals having been vaccinated against H-RSV.

In a further aspect, the invention relates to a method for MHC class II haplotype specific vaccination of a subject against H-RSV, the method comprising

the steps of: (a) determining the MHC class II haplotype of the subject; and, (b)
administering to the subject a pharmaceutical composition comprising a peptide
comprising at least 9, 10, 11, or 12 contiguous amino acids from an amino acid
sequence selected from Table 1, whereby the amino acid sequence matches the

5   MHC class II haplotype of the subject in accordance with Table 1. In this method,
the MHC class II haplotype of the subject is determined by known methods as
described above. Method for preparing the peptides to be used in this method, as
well as pharmaceutical composition comprising these peptides and methods for their
preparation are described herein below. In a preferred method of vaccination, a

10 pharmaceutical composition suitable for parenteral administration is administered
parenterally, a pharmaceutical composition suitable for transdermal administration is
administered transdermally, or a pharmaceutical composition suitable for inhalation
is inhaled.

In yet another aspect, the invention relates to the use of a peptide comprising

15 at least 9, 10, 11, or 12 contiguous amino acids from an amino acid sequence
selected from Table 1, for the manufacture of a vaccine for MHC class II haplotype
specific prophylaxis or therapy of H-RSV infection in a subject, whereby the amino
acid sequence matches the MHC class II haplotype of the subject in accordance with
Table 1. Preferably, the vaccine is a pharmaceutical composition suitable for

20 parenteral or transdermal administration.

Peptides for use in the methods of the invention

The peptides of the invention contain an epitope that specifically recognised
by MHC class II molecules in accordance with Table 1. The peptides of the

25 invention thus bind the groove or cleft of the MHC class II molecule in question.
The peptides of the invention typically comprise at least about 9, 10, 11, 12, 15, or
18 residues. In certain embodiments the peptides will not exceed about 150, 100 or
50 residues and typically will not exceed about 20 residues. Thus, a wide range of
peptide sizes may be used in the present invention.

30 Particularly because the peptides to be used in the methods of the invention are
relatively short, the peptides can be readily synthesised using known methods. For
example, the peptides can be synthesised by the well-known Merrifield solid-phase

synthesis method in which amino acids are sequentially added to a growing chain.
See Merrifield (1963), *J. Am. Chem. Soc.* 85:2149-2156; and Atherton et al., "Solid
Phase Peptide Synthesis," IRL Press, London, (1989). Automatic peptide
synthesisers are commercially available from numerous suppliers, such as Applied

5   Biosystems, Foster City, California. Additional synthetic approaches for preparing
the peptides of the invention are described in the Examples herein.

        Alternatively, the F and G protein-derived peptides may be prepared as part of
larger polypeptides comprising one or more of the peptides of the invention. Such
larger polypeptides be prepared using well-known recombinant techniques in which

10   a nucleotide sequence encoding the polypeptide of interest is expressed in cultured
cells such as described in Ausubel et al., Current Protocols in Molecular Biology,
Greene Publishing and Wiley-Interscience, New York (1987) and in Sambrook and
Russell (2001) "Molecular Cloning: A Laboratory Manual (3rd edition), Cold Spring
Harbor Laboratory, Cold Spring Harbor Laboratory Press, New York, both of which

15   are incorporated herein by reference in their entirety. Also see, Kunkel, 1985, Proc.
Natl. Acad. Sci. 82:488 (describing site directed mutagenesis) and Roberts *et al.*,
1987, Nature 328:731-734 or Wells, J.A., et al. (1985) Gene 34:315 (describing
cassette mutagenesis).

        Typically, nucleic acids encoding the desired polypeptides are used in

20   expression vectors. The phrase "expression vector" generally refers to nucleotide
sequences that are capable of affecting expression of a gene in hosts compatible with
such sequences. These expression vectors typically include at least suitable promoter
sequences and optionally, transcription termination signals. Additional factors
necessary or helpful in effecting expression may also be used as described herein.

25   DNA encoding the polypeptides of the present invention will typically be
incorporated into DNA constructs capable of introduction into and expression in an
in vitro cell culture. Specifically, DNA constructs will be suitable for replication in a
prokaryotic host, such as bacteria, *e.g.*, *E. coli*, or may be introduced into a cultured
mammalian, plant, insect, yeast, fungi or other eukaryotic cell lines.

30   DNA constructs prepared for introduction into a particular host will typically
include a replication system recognised by the host, the intended DNA segment
encoding the desired polypeptide, and transcriptional and translational initiation and

termination regulatory sequences operably linked to the polypeptide encoding
segment. A DNA segment is "operably linked" when it is placed into a functional
relationship with another DNA segment. For example, a promoter or enhancer is
operably linked to a coding sequence if it stimulates the transcription of the

5   sequence. DNA for a signal sequence is operably linked to DNA encoding a
polypeptide if it is expressed as a preprotein that participates in the secretion of the
polypeptide. Generally, DNA sequences that are operably linked are contiguous,
and, in the case of a signal sequence, both contiguous and in reading phase.
However, enhancers need not be contiguous with the coding sequences whose

10   transcription they control. Linking is accomplished by ligation at convenient
restriction sites or at adapters or linkers inserted in lieu thereof.

The selection of an appropriate promoter sequence generally depends upon the
host cell selected for the expression of the DNA segment. Examples of suitable
promoter sequences include prokaryotic, and eukaryotic promoters well-known in

15   the art. See, e.g., Sambrook and Russell (2001, *supra*). The transcriptional
regulatory sequences will typically include a heterologous enhancer or promoter
which is recognised by the host. The selection of an appropriate promoter will
depend upon the host, but promoters such as the trp, lac and phage promoters, tRNA
promoters and glycolytic enzyme promoters are known and available. See, e.g.,

20   Sambrook and Russell (2001, *supra*).

Conveniently available expression vectors which include the replication
system and transcriptional and translational regulatory sequences together with the
insertion site for the polypeptide encoding segment may be employed. Examples of
workable combinations of cell lines and expression vectors are described in

25   Sambrook and Russell (2001, *supra*). For example, suitable expression vectors may
be expressed in, e.g., insect cells, e.g., Sf9 cells, mammalian cells, e.g., CHO cells
and bacterial cells, e.g., *E. coli*.

It will be understood that the H-RSV F or G protein derived (poly)peptides of
the invention may be modified to provide a variety of desired attributes, e.g.,

30   improved pharmacological characteristics, while increasing or at least retaining
substantially all of the biological activity of the unmodified peptide. For instance,
the peptides can be modified by extending, decreasing the amino acid sequence of

the peptide. Substitutions with different amino acids or amino acid mimetics can also be made.

The individual residues of the immunogenic H-RSV F or G protein derived (poly)peptides of the invention can be incorporated in the peptide by a peptide bond or peptide bond mimetic. A peptide bond mimetic of the invention includes peptide backbone modifications well known to those skilled in the art. Such modifications include modifications of the amide nitrogen, the α-carbon, amide carbonyl, complete replacement of the amide bond, extensions, deletions or backbone cross-links. See, generally, Spatola, Chemistry and Biochemistry of Amino Acids, Peptides and Proteins, Vol. VII (Weinstein ed., 1983). Several peptide backbone modifications are known, these include, ψ [CH$_2$S], ψ [CH$_2$NH], ψ [CSNH$_2$], ψ [NHCO], ψ [COCH$_2$] and ψ [(E) or (Z) CH=CH]. The nomenclature used above, follows that suggested by Spatola, above. In this context, ψ indicates the absence of an amide bond. The structure that replaces the amide group is specified within the brackets.

Amino acid mimetics may also be incorporated in the peptides. An "amino acid mimetic" as used here is a moiety other than a naturally occurring amino acid that conformationally and functionally serves as a substitute for an amino acid in a peptide of the present invention. Such a moiety serves as a substitute for an amino acid residue if it does not interfere with the ability of the peptide to elicit an immune response against the appropriate H-RSV F or G protein derived epitope. Amino acid mimetics may include non-protein amino acids, such as β-, γ-, δ-amino acids, β-, γ-, δ--imino acids (such as piperidine-4-carboxylic acid) as well as many derivatives of L-α-amino acids. A number of suitable amino acid mimetics are known to the skilled artisan, they include cyclohexylalanine, 3-cyclohexylpropionic acid, L-adamantyl alanine, adamantylacetic acid and the like. Peptide mimetics suitable for peptides of the present invention are discussed by Morgan and Gainor, (1989) Ann. Repts. Med. Chem. 24:243-252.

As noted above, the peptides employed in the subject invention need not be identical, but may be substantially identical, to the corresponding amino acid sequence of the H-RSV F or G protein derived epitope as listed in Table 1. Therefore, the peptides may be subject to various changes, such as insertions, deletions, and substitutions, either conservative or non-conservative, where such

changes might provide for certain advantages in their use. The peptides of the invention can be modified in a number of ways so long as they comprise a sequence substantially identical (as defined below) to an amino acid sequence of an H-RSV F or G protein derived epitope as listed in Table 1.

5   Alignment and comparison of relatively short amino acid sequences (less than about 30 residues) is typically straightforward. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for

10   similarity method of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. (USA) 85:2444, by computerised implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by inspection, and the best alignment (i.e., resulting in the highest percentage of sequence similarity

15   over the comparison window) generated by the various methods is selected.

The term "sequence identity" means that two polypeptide sequences are identical (i.e., on an amino acid-by-amino acid basis) over a window of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the

20   number of positions at which the identical residues occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

As applied to the peptides of the invention, the term "substantial identity"

25   means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at least 95 percent sequence identity or more (e.g., 99 percent sequence identity). Preferably, residue positions which are not identical differ by conservative amino acid

30   substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of

amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of
amino acids having amide-containing side chains is asparagine and glutamine; a
group of amino acids having aromatic side chains is phenylalanine, tyrosine, and
tryptophan; a group of amino acids having basic side chains is lysine, arginine, and

5   histidine; and a group of amino acids having sulphur-containing side chains is
cysteine and methionine. Preferred conservative amino acids substitution groups are:
valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine,
and asparagine-glutamine.

10   Pharmaceutical (vaccine) compositions and their administration
     The peptides of the present invention and pharmaceutical compositions thereof
are useful for administration to mammals, particularly humans, to treat and/or
prevent H-RSV infection as well as LRT and other diseases caused by H-RSV
infection. Suitable formulations are found in Remington's Pharmaceutical Sciences,

15   Mack Publishing Company, Philadelphia, Pa., 17th ed. (1985), which is incorporated
herein by reference.
     The immunogenic peptides of the invention are administered prophylactically
or to an individual already suffering from the disease. The compositions are
administered to a patient in an amount sufficient to elicit an effective immune

20   response to the MHC molecule from which the peptides are derived. An amount
adequate to accomplish this is defined as "therapeutically effective dose" or
"immunogenically effective dose." Amounts effective for this use will depend on,
e.g., the peptide composition, the manner of administration, the stage and severity of
the disease being treated, the weight and general state of health of the patient, and

25   the judgement of the prescribing physician, but generally range for the initial
immunisation (that is for therapeutic or prophylactic administration) from about 0.1
mg to about 1.0 mg per 70 kilogram patient, more commonly from about 0.5 mg to
about 0.75 mg per 70 kg of body weight. Boosting dosages are typically from about
0.1 mg to about 0.5 mg of peptide using a boosting regimen over weeks to months

30   depending upon the patient's response and condition. A suitable protocol would
include injection at time 0, 2, 6, 10 and 14 weeks, followed by booster injections at
24 and 28 weeks.

The pharmaceutical compositions are intended for parenteral, oral or transdermal administration. Preferably, the pharmaceutical compositions are administered parenterally, e.g., subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for parenteral administration which

5   comprise a solution of the immunogenic peptides dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilised by conventional, well known sterilisation techniques, or may be sterile filtered. The resulting aqueous solutions

10   may be packaged for use as is, or lyophilised, the lyophilised preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride,

15   potassium chloride, calcium chloride, sorbitan monolaurate, and triethanolamine oleate.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose,

20   magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more peptides of the invention, and more preferably at a concentration of 25%-75%. As noted above, the compositions are

25   intended to induce an immune response to the peptides. Thus, compositions and methods of administration suitable for maximising the immune response are preferred. For instance, peptides may be introduced into a host, including humans, linked to a carrier or as a homopolymer or heteropolymer of active peptide units. Alternatively, the a "cocktail" of peptides can be used. A mixture of more than one

30   peptide has the advantage of increased immunological reaction and, where different peptides are used to make up the polymer, the additional ability to induce antibodies to a number of epitopes. For instance, peptides comprising sequences from

16

hypervariable regions of α and β chains may be used in combination. Useful carriers are well known in the art, and include, e.g., thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly(lysine:glutamic acid), influenza, hepatitis B virus core protein, hepatitis B virus recombinant vaccine and

5   the like.

The compositions preferably also include an adjuvant. A number of adjuvants are well known to one skilled in the art. Suitable adjuvants include incomplete Freund's adjuvant, alum, aluminum phosphate, aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-

10   isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn -glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion.

15   The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against the immunogenic peptide. A particularly useful adjuvant and immunisation schedule are described in Kwak et al. New Eng. J. Med. 327-1209-1215 (1992), which is incorporated herein by reference. The immunological adjuvant described there comprises 5% (wt/vol) squalene, 2.5% Pluronic L121

20   polymer and 0.2% polysorbate in phosphate buffered saline.

The concentration of immunogenic peptides of the invention in the pharmaceutical formulations can vary widely, i.e. from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the

25   particular mode of administration selected.

The peptides of the invention can also be expressed by attenuated viral hosts, such as vaccinia or fowlpox. This approach involves the use of vaccinia virus as a vector to express nucleotide sequences that encode the peptides of the invention. Upon introduction into a host, the recombinant vaccinia virus expresses the

30   immunogenic peptide, and thereby elicits an immune response. Vaccinia vectors and methods useful in immunisation protocols are described in, e.g., U.S. Pat. No. 4,722,848, incorporated herein by reference. Another vector is BCG (Bacille

Calmette Guerin). BCG vectors are described in Stover et al. (Nature 351:456-460
(1991)) which is incorporated herein by reference. A wide variety of other vectors
useful for therapeutic administration or immunisation of the peptides of the
invention, e.g., Salmonella typhi vectors and the like, will be apparent to those
5   skilled in the art from the description herein.

        Further guidance regarding formulations that are suitable for various types of
administration can be found in *Remington's Pharmaceutical Sciences*, Mace
Publishing Company, Philadelphia, PA, 17th ed. (1985). For a brief review of
methods for drug delivery, *see*, Langer, *Science* 249:1527-1533 (1990). Both of
10   these references are incorporated herein by reference in their entirety. E.g.
transdermal delivery systems include patches, gels, tapes and creams, and can
contain excipients such as solubilisers, permeation enhancers (e.g. fatty acids, fatty
acid esters, fatty alcohols and amino acids), hydrophilic polymers (e.g.
polycarbophil and polyvinyl pyrillidine and adhesives and tackifiers (e.g.
15   polyisobutylenes, silicone-based adhesives, acrylates and polybutene). Transmucosal
delivery systems include patches, tablets, suppositories, pessaries, gels, and creams,
and can contain excipients such as solubilisers and enhancers (e.g. propylene glycol,
bile salts and amino acids), and other vehicles (e.g. polyethylene glycol, fatty acid
esters and derivatives, and hydrophilic polymers such as hydroxypropylmethyl
20   cellulose and hyaluronic acid). Injectable delivery systems include solutions,
suspensions, gels, microspheres and polymeric injectables, and can comprise
excipients such as solubility-altering agents (e.g. ethanol, propylene glycol and
sucrose) and polymers (e.g. polycaprylactones, and PLGA's). Implantable systems
include rods and discs, and can contain excipients such as PLGA and polycapryl
25   lactone. Other delivery systems that can be used for administering the
pharmaceutical composition of the invention include intranasal delivery systems
such as sprays and powders, sublingual delivery systems and systems for delivery by
inhalation. For administration by inhalation, the pharmaceutical compositions of the
present invention are conveniently delivered in the form of an aerosol spray
30   presentation from pressurised packs or a nebuliser, with the use of a suitable
propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane,
dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a

pressurised aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the peptides of the invention and a suitable powder base such as lactose or starch. The

5   pharmaceutical compositions of the invention may be further formulated for administration by inhalation as e.g. described in U.S. Patent No. 6,358,530.

In another aspect the invention relates to a method for producing a pharmaceutical composition comprising the (poly)peptides of the invention. The method comprises at least the steps of mixing the (poly)peptides of the invention

10   obtained in the methods described above with a pharmaceutically acceptable carrier and further constituents like adjuvant as described above.

19

Examples

1. Material and methods

1.1. Peripheral blood mononuclear cells (PBMC's)

Buffycoats were obtained from healthy adult blood donors with informed

5   consent. PBMC's were isolated by density centrifugation using lymphoprep

(Nycomed Pharma). PBMC's were either thawed from cryopreserved samples (–

135°C), in RPMI 1640 (Gibco), 10% Dimethyl Sulfoxide, 30% fetal bovine serum

(FBS, Gibco), or used fresh.

1.2 Virus and peptides

10   Human RSV strain A2 (available under number VR 1302 from the ATCC,

Manassas, VA, USA) was propagated in HEp-2 cells and titrated by plaque assay.

The virus was routinely used in experiments at a MOI of 1 (MOI = multiplicity of

infection). A series of 94 peptides, 18 amino acid residues long was synthesised by

standard solid phase Fmoc chemistry. All peptides were analysed by mass

15   spectrometry and gave the anticipated $(M+H)^+$ except for peptides 11,12 and 85, that

were excluded from the study. Peptides 69 and 70 were made at the end of the series

and were numbered 95 and 96 respectively. The peptides synthesised were based on

the sequence of the fusion protein F of RSV strain A2. They overlapped by 12

amino acid residues. For the G protein we used a series over overlapping peptides

20   representing amino acid residues 161-199 of the G protein of RSV strain A2. The

sequences of these G protein-derived peptides were: DFHFEVFNFVPCSI;

FEVFNFVPCSICSNN; FNFVPCSICSNNPTCW; SICSNNPTCWAICKRI;

SNNPTCWAICKRIPNKKP; WAICKRIPNKKPGKK; and ICKRIPNKKPGKKTT.

1.3 Elispot assay

25

96 well filtration plates (MAIPS4510, Millipore) were coated overnight with

anti-IFN-γ coating antibody (Ab) 1-D1K (100μl, 15μg/ml, Mabtech) in 0.1M

carbonate-bicarbonate buffer pH 9.6, at 4°C. Before adding the cells the plate were

washed thoroughly with PBS, blocked for one hour at 37°C with RPMI 1640

30   containing 10% FBS. Cells and either virus or peptide were added to the well in a

final volume of 200 μl of RPMI 1640, 10% FBS, penicillin and streptomycin. Cells

were incubated at 37°C overnight in a humidified incubator. Then cells were

removed by thoroughly washing in PBS and 100 μl of detecting mAb 7-B6-1-biotin
(Mabtech), diluted to 1 μg/ml in PBS-0.5% FBS was added to the wells. After
incubating 2 hrs at room temperature and washes in PBS, 100 μl (1/1000 diluted in
PBS, 0.5% FBS) ExtraAvidine Alkaline Phosphatase Conjugate (Sigma) was added

5   and incubated 1-2 hours at room temperature. Then the plates were washed in PBS
and BCIP/NBT substrate was added 100 μl/well, (BCIP/NBT tablets Sigma, 1 tablet
diluted in 10ml H_2O). Spots were counted by two investigators. Most data are
represented as the number of spots per 10^{-6} PBMC minus background of
unstimulated wells. Only in Table 1 the uncorrected number of spots is shown. In

10   the mAb blocking experiments the antibodies were added to the cells prior to the
stimulating peptides. The cells and mAb were preincubated for 30 minutes at 37°C,
after which the peptides were added to the cultures. The mAb used are culture
supernatants of hybridoma's B8.11.2 producing anti HLA-DR and SPVL3 anti
HLA-DQ.

15

2.   Immunodominancy of F protein derived peptides

A set of overlapping peptides 18 amino acid residues long and overlapping by
12 amino acid residues, spanning the entire length of the F protein of RSV strain A2
was synthesised. In a preliminary search for antigenic epitopes pools of 6

20   consecutive peptides were made. These peptide pools were analysed in an elispot
assay for their ability to induce IFN-γ production in PBMC from healthy donors. In
Table 1 the data are summarised for a group of donors in which the most frequently
occurring MHC class II molecules within the caucasian population are represented.
We were able to detect IFN-γ production in response to stimulation with intact RSV

25   at a MOI of 1. The level of the response varied from donor to donor but was
generally in the order of 100 to 500 spots per 10^{-6} PBMC. There was no correlation
between better responses and the time of the year the blood was sampled. Most
donors responded to more than one peptide pool. Pool four was not recognised by
any donor. The peptides represented in this pool cover a stretch of 46 amino acid

30   residues from 121 to 168, partly overlapping the 27 amino acid residue peptide (110-
136) that is removed from the fusion protein after furin cleavage in the Golgi-

compartment (Gonzalez-Ryes et al., 2001, Proc. Natl. Acad. Sci. USA 98: 9859-
9864).

In a second step the individual peptides from the positive pools that were able
to induce IFN-γ production were identified for five individual donors (data not

5   shown). For most pools a clearcut response identified one or two positive peptides.
Sometimes two adjacent peptides were found positive that possibly contained an
overlapping epitope. The peptide length of 18 amino acid residues is sufficiently
long to encompass MHC class II peptides and less suitable for MHC class I
interaction. For presentation by MHC class I molecules the 18-mer peptides require

10   cleavage to 9-11 amino acid residues in order to be able to bind into the class I
binding groove. Yet in our initial screens we used fairly high concentrations of
peptides that had not been purified by HPLC. Therefore, it could be possible that the
responses we observed were either CD4 or CD8 T cell responses. To determine the
T cell subset –CD4 or CD8- responding to the peptides, we performed elispot assays

15   using PBMC depleted for either CD8 T cells or CD4 T cells. For all the peptides that
we have tested with subset depleted PBMC we found that the responses where
completely abrogated after CD4 depletion (data not shown). The CD4 involvement
in the peptide specific responses was further confirmed by peptide stimulations of
PBMC in the presence of HLA DR blocking antibody (Ab) B8.11.2 or HLA DQ

20   blocking Ab SPVL3 (data not shown). For most peptides the number of IFN-γ
producing cells dropped to zero when the peptide concentration was titrated in the
presence of MHC class II blocking Ab B8.11.2 (data not shown). However, in two
donors with HLA type DRB1*1302, DR15, DQ6 and DRB1*1301, DQ6 the
response against peptides 50 and 51 is DQ restricted (data not shown). These

25   peptides are also recognised in a DQ restricted fashion by a donor expressing HLA
alleles DR1, DQ5 (data not shown). A third donor (HLA DR15,9 DQ3,6) recognised
two adjacent peptides (peptide numbers 45,46) also in the context of HLA-DQ (data
not shown). Another observation made from these experiments is that there appeared
to be large differences in the minimal peptide concentration (40 nM - 5 μM)

30   necessary for optimal T cell activation for the different individual peptides. Yet the
fact that we observed the responses means that during a natural infection the total
array of peptides that we have detected in the present series of tests can be

functionally presented to the immune system, since we could pick up the response

without pre-expansion of the T cells in a direct elispot assay. To determine the MHC

restriction element for a final set of peptides recognised by donors carrying HLA-

DR7,11,15 alleles, antigen presenting cells (APC) were depleted from high

5   responder heterozygous donors. Elispot assays were performed using these APC-

depleted PBMC as responders and fixed PBMC from other homozygous donors as

APC. It was found that HLA-DR7 presents peptides 6, 10, 39 and 91 and HLA-

DR11 presents peptide 39 to CD4 cells.

From the data obtained in the second step described above, we selected a set of

10   30 peptides that were recognised by at least one donor. In Table 1 we summarise the

T cell responses against this set of F peptides for a series of donors in which the

most frequently occurring HLA-DR types within the caucasian population are

represented (Knipper et al., 2000, Human Immunol. 61: 605-614). For some

peptides the possible P1 anchor residue is marked, based on known antigen binding

15   motifs or computer algorithms (Sturniolo et al., 1999, Nature Biotech. 17: 555-561;

Rammensee et al., 1997, Molecular Biology Intelligence Unit, MHC ligands and

peptide motifs, Springer Verlag, Heidelberg Germany, Int. ISBN 3-540-63125-9).

3.   Immunodominancy of G protein derived peptides

20   We used the same procedure that was employed for the characterisation of

immunodominant peptides within the Fusion protein of RSV to characterise

immunodominant peptides derived from the G protein of RSV. Because of the high

variability of the G protein in different virus isolates we focussed our analysis on a

conserved region (amino acid residues 161-199) within G that possibly plays a role

25   in viral attachment to the cell. Within this region we have characterised two peptides

(see table 1) that can be recognised by CD4 T cells present in PBMC samples of

healthy blood donors.

Table 1

MHC haplotype	Peptide number	RSV protein	Aa position	SEQ ID No.	Amino Acid sequence
HLA-DR1	2	F	7-24	3	KANAITILTAVTFCFAS
	3		13-30	4	TILTAVTFCFASGQNITE
	40		235-252	12	REFSVNAGVTTPVSTYML
	77		457-474	20	YYVNKQEGKSLYVKGEPI
HLA-DQ5	50		295-312	15	EVLAYVVQLPLYGVIDTP
	51		301-318	16	VQLPLYGVIDTPCWKLHT
HLA-DR4 (DRB1*0401)	2	F	7-24	3	KANAITILTAVTFCFAS
	5		25-42	5	GQNITEEFYQSTCSAVSK
	6		31-48	6	EFYQSTCSAVSKGYLSAL
	14		79-96	8	IKQELDKYKNAVTELQLL
	15		85-102	9	KYKNAVTELQLLMQSTPP
	66		391-408	18	YDCKIMTSKTDVSSSVIT
	72		427-444	19	KNRGIIKTFSNGCDYVSN
HLA-DR7	5	F	25-42	5	GQNITEEFYQSTCSAVSK
	6		31-48	6	EFYQSTCSAVSKGYLSAL
	10		55-72	7	SVITIELSNIKENKCNGT
	15		85-102	9	KYKNAVTELQLLMQSTPP
	30		175-192	10	NKAVVSLSNGVSVLTSKV
	39		229-246	11	RLLEITREFSVNAGVTTP
	95		409-426	22	SLGAIVSCYGKTKCTASN
	91		541-558	21	LIAVGLLLYCKARSTPVT
HLA-DR9	45	F	265-282	13	PITNDQKKLMSNNVQIVR
	46		271-288	14	KLMSNNVQIVRQQSYSI
HLA-DR11	39	F	229-246	11	RLLEITREFSVNAGVTTP
	57		337-354	17	TDRGWYCDNAGSVSFFPQ
HLA-DR13 (DRB1*1301)	57	F	337-354	17	TDRGWYCDNAGSVSFFPQ
HLA-DQ6	50		295-312	15	EVLAYVVQLPLYGVIDTP
	51		301-318	16	VQLPLYGVIDTPCWKLHT
HLA-DR15,51	14	F	79-96	8	IKQELDKYKNAVTELQLL
	95		409-426	22	SLGAIVSCYGKTKCTASN
HLA-DPw4 (DPB1*0401) and (DPB1*0402)		G	162-175	23	DFHFEVFNFVPCSI
HLA-DR		G	177-194	24	SNNPTCWAICKRIPNKKP

SEQUENCE LISTING

    Met Glu Leu Leu Ile Leu Lys Ala Asn Ala Ile Thr Thr Ile Leu Thr
40 1   5   10   15

    Ala Val Thr Phe Cys Phe Ala Ser Gly Gln Asn Ile Thr Glu Glu Phe
                20   25   30
45

    Tyr Gln Ser Thr Cys Ser Ala Val Ser Lys Gly Tyr Leu Ser Ala Leu
            35   40   45

50
    Arg Thr Gly Trp Tyr Thr Ser Val Ile Thr Ile Glu Leu Ser Asn Ile
        50   55   60

55 Lys Glu Asn Lys Cys Asn Gly Thr Asp Ala Lys Val Lys Leu Ile Lys
        65   70   75   80

25

```
      Gln Glu Leu Asp Lys Tyr Lys Asn Ala Val Thr Glu Leu Gln Leu Leu
                      85              90              95

  5   Met Gln Ser Thr Pro Pro Thr Asn Asn Arg Ala Arg Arg Glu Leu Pro
                     100             105             110

      Arg Phe Met Asn Tyr Thr Leu Asn Asn Ala Lys Lys Thr Asn Val Thr
 10              115             120             125

      Leu Ser Lys Lys Arg Lys Arg Arg Phe Leu Gly Phe Leu Leu Gly Val
              130             135             140
 15

      Gly Ser Ala Ile Ala Ser Gly Val Ala Val Ser Lys Val Leu His Leu
      145             150             155             160
 20
      Glu Gly Glu Val Asn Lys Ile Lys Ser Ala Leu Leu Ser Thr Asn Lys
                      165             170             175

 25   Ala Val Val Ser Leu Ser Asn Gly Val Ser Val Leu Thr Ser Lys Val
                      180             185             190

      Leu Asp Leu Lys Asn Tyr Ile Asp Lys Gln Leu Leu Pro Ile Val Asn
 30              195             200             205

      Lys Gln Ser Cys Ser Ile Ser Asn Ile Glu Thr Val Ile Glu Phe Gln
              210             215             220
 35

      Gln Lys Asn Asn Arg Leu Leu Glu Ile Thr Arg Glu Phe Ser Val Asn
      225             230             235             240
 40
      Ala Gly Val Thr Thr Pro Val Ser Thr Tyr Met Leu Thr Asn Ser Glu
                      245             250             255

 45   Leu Leu Ser Leu Ile Asn Asp Met Pro Ile Thr Asn Asp Gln Lys Lys
                      260             265             270

      Leu Met Ser Asn Asn Val Gln Ile Val Arg Gln Gln Ser Tyr Ser Ile
 50              275             280             285

      Met Ser Ile Ile Lys Glu Glu Val Leu Ala Tyr Val Val Gln Leu Pro
              290             295             300
 55

      Leu Tyr Gly Val Ile Asp Thr Pro Cys Trp Lys Leu His Thr Ser Pro
      305             310             315             320
```

```
    Leu Cys Thr Thr Asn Thr Lys Glu Gly Ser Asn Ile Cys Leu Thr Arg
                  325                 330                 335
5


    Thr Asp Arg Gly Trp Tyr Cys Asp Asn Ala Gly Ser Val Ser Phe Phe
                  340                 345                 350
10
    Pro Gln Ala Glu Thr Cys Lys Val Gln Ser Asn Arg Val Phe Cys Asp
                  355                 360                 365


15  Thr Met Asn Ser Leu Thr Leu Pro Ser Glu Ile Asn Leu Cys Asn Val
                  370                 375                 380


    Asp Ile Phe Asn Pro Lys Tyr Asp Cys Lys Ile Met Thr Ser Lys Thr
20  385                 390                 395                 400


    Asp Val Ser Ser Ser Val Ile Thr Ser Leu Gly Ala Ile Val Ser Cys
                      405                 410                 415
25
    Tyr Gly Lys Thr Lys Cys Thr Ala Ser Asn Lys Asn Arg Gly Ile Ile
                  420                 425                 430


30
    Lys Thr Phe Ser Asn Gly Cys Asp Tyr Val Ser Asn Lys Gly Met Asp
                  435                 440                 445


35  Thr Val Ser Val Gly Asn Thr Leu Tyr Tyr Val Asn Lys Gln Glu Gly
                  450                 455                 460


    Lys Ser Leu Tyr Val Lys Gly Glu Pro Ile Ile Asn Phe Tyr Asp Pro
40  465                 470                 475                 480


    Leu Val Phe Pro Ser Asp Glu Phe Asp Ala Ser Ile Ser Gln Val Asn
                  485                 490                 495
45
    Glu Lys Ile Asn Gln Ser Leu Ala Phe Ile Arg Lys Ser Asp Glu Leu
                  500                 505                 510


50
    Leu His Asn Val Asn Ala Gly Lys Ser Thr Thr Asn Ile Met Ile Thr
                  515                 520                 525


55  Thr Ile Ile Ile Val Ile Ile Val Ile Leu Leu Ser Leu Ile Ala Val
                  530                 535                 540
```

```
        Gly Leu Leu Leu Tyr Cys Lys Ala Arg Ser Thr Pro Val Thr Leu Ser
        545             550             555             560


  5     Lys Asp Gln Leu Ser Gly Ile Asn Asn Ile Ala Phe Ser Asn
                        565             570



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        <211>  306

        <212>  PRT

 15     <213>  respiratory syncytial virus



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 25     Trp Asp Thr Leu Asn His Leu Leu Phe Ile Ser Ser Cys Leu Tyr Lys
                    20              25              30


        Leu Asn Leu Lys Ser Val Ala Gln Ile Thr Leu Ser Ile Leu Ala Met
 30             35              40              45


        Ile Ile Ser Thr Ser Leu Ile Ile Ala Ala Ile Ile Phe Ile Ala Ser
            50              55              60
 35

        Ala Asn His Lys Val Thr Pro Thr Thr Ala Ile Ile Gln Asp Ala Thr
        65              70              75              80
 40

        Ser Gln Ile Lys Asn Thr Thr Pro Thr Tyr Leu Thr Gln Asn Pro Gln
                        85          .        90              95


 45     Leu Gly Ile Ser Pro Ser Asn Pro Ser Glu Ile Thr Ser Gln Ile Thr
                    100             105             110


        Thr Ile Leu Ala Ser Thr Thr Pro Gly Val Lys Ser Thr Leu Gln Ser
 50             115             120             125


        Thr Thr Val Lys Thr Lys Asn Thr Thr Thr Thr Gln Thr Gln Pro Ser
                130             135             140
 55

        Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Ser Lys Pro Asn
        145             150             155             160
```

```
Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys
            165             170             175

Ser Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys
            180             185             190

Lys Pro Gly Lys Lys Thr Thr Thr Lys Pro Thr Lys Lys Pro Thr Leu
            195             200             205

Lys Thr Thr Lys Lys Asp Pro Lys Pro Gln Thr Thr Lys Ser Lys Glu
            210             215             220

Val Pro Thr Thr Lys Pro Thr Glu Glu Pro Thr Ile Asn Thr Thr Lys
225             230             235             240

Thr Asn Ile Ile Thr Thr Leu Leu Thr Ser Asn Thr Thr Gly Asn Pro
            245             250             255

Glu Leu Thr Ser Gln Met Glu Thr Phe His Ser Thr Ser Ser Glu Gly
            260             265             270

Asn Pro Ser Pro Ser Gln Val Ser Thr Thr Ser Glu Tyr Pro Ser Gln
            275             280             285

Pro Ser Ser Pro Pro Asn Thr Pro Arg Gln Ser Pro Pro Asn Thr Pro
            290             295             300

Arg Gln
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       Ser Lys

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Gly Thr

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Leu Leu

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<212> PRT

<213> respiratory syncytial virus

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5   Pro Pro

10   <210> 10

     <211> 18

     <212> PRT

15   <213> respiratory syncytial virus

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     1               5               10              15
```

25   Lys Val

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30
     <211> 18

     <212> PRT

35   <213> respiratory syncytial virus

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     1               5               10              15
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45   Thr Pro

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     <211> 18

     <212> PRT

55   <213> respiratory syncytial virus

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Met Leu

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Ile

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<211> 18

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Glu Val Leu Ala Tyr Val Val Gln Leu Pro Leu Tyr Gly Val Ile Asp
1               5                   10                  15

Thr Pro


<210>  16

<211>  18

<212>  PRT

<213>  respiratory syncytial virus


<400>  16

Val Gln Leu Pro Leu Tyr Gly Val Ile Asp Thr Pro Cys Trp Lys Leu
1               5                   10                  15

His Thr


<210>  17

<211>  18

<212>  PRT

<213>  respiratory syncytial virus


<400>  17

Thr Asp Arg Gly Trp Tyr Cys Asp Asn Ala Gly Ser Val Ser Phe Phe
1               5                   10                  15

Pro Gln


<210>  18

<211>  18

<212>  PRT
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34

<213>   respiratory syncytial virus

5   <400>   18

       Tyr Asp Cys Lys Ile Met Thr Ser Lys Thr Asp Val Ser Ser Ser Val
       1   5   10   15

10
       Ile Thr

15   <210>   19

       <211>   18

       <212>   PRT
20
       <213>   respiratory syncytial virus

25   <400>   19

       Lys Asn Arg Gly Ile Ile Lys Thr Phe Ser Asn Gly Cys Asp Tyr Val
       1   5   10   15

30
       Ser Asn

35   <210>   20

       <211>   18

       <212>   PRT
40
       <213>   respiratory syncytial virus

45   <400>   20

       Tyr Tyr Val Asn Lys Gln Glu Gly Lys Ser Leu Tyr Val Lys Gly Glu
       1   5   10   15

50
       Pro Ile

55   <210>   21

       <211>   18

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<212>  PRT

<213>  respiratory syncytial virus


<400>  21

Leu Ile Ala Val Gly Leu Leu Leu Tyr Cys Lys Ala Arg Ser Thr Pro
1                5                   10                  15

Val Thr


<210>  22

<211>  18

<212>  PRT

<213>  respiratory syncytial virus


<400>  22

Ser Leu Gly Ala Ile Val Ser Cys Tyr Gly Lys Thr Lys Cys Thr Ala
1                5                   10                  15

Ser Asn


<210>  23

<211>  14

<212>  PRT

<213>  respiratory syncytial virus


<400>  23

Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile
1                5                   10


<210>  24

<211>  18

<212>  PRT
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<213> respiratory syncytial virus

<400> 24

Ser Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys
1   5   10   15

Lys Pro

<210> 25

<211> 15

<212> PRT

<213> respiratory syncytial virus

<400> 25

Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Ser Asn Asn
1   5   10   15

<210> 26

<211> 16

<212> PRT

<213> respiratory syncytial virus

<400> 26

Phe Asn Phe Val Pro Cys Ser Ile Cys Ser Asn Asn Pro Thr Cys Trp
1   5   10   15

<210> 27

<211> 16

<212> PRT

<213> respiratory syncytial virus

<400> 27

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Ser Ile Cys Ser Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile
1               5                   10                  15
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<210> 28

<211> 15

<212> PRT

<213> respiratory syncytial virus

<400> 28

```
Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys Lys Pro Gly Lys Lys
1               5                   10                  15
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<210> 29

<211> 15

<212> PRT

<213> respiratory syncytial virus

<400> 29

```
Ile Cys Lys Arg Ile Pro Asn Lys Lys Pro Gly Lys Lys Thr Thr
1               5                   10                  15
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